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Claims for the following Contracting State: ES.

(54) **Stable aqueous thrombin solution.**

(57) A stable aqueous thrombin solution contains thrombin and, as stabilizer, a mixture of a sugar and an amino acid.

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Description

STABLE AQUEOUS THROMBIN SOLUTION

This invention relates to a stable aqueous thrombin solution.

Thrombin acts on fibrinogen to form fibrin, thereby exerting a blood coagulating effect. For this reason, thrombin is used clinically as a hemostatic agent for topical application in the field of surgery.

Since thrombin is very unstable in its aqueous solution, it is usually made into a lyophilized medical preparation, which is used after being dissolved at the time of use.

However, liquid preparations are advantageous as compared with the lyophilized preparations in that they can be easily administered without being dissolved in distilled water or other solvents for injection.

From such a viewpoint, there have been proposed in recent years various methods for preparing a stable aqueous liquid composition of thrombin. For example, Japanese Patent Application Kokai (Laid-Open) No. 56-39,782 proposes a method comprising using an organic carboxylic acid, Japanese Patent Application Kokai (Laid-Open) No. 57-18,985 proposes a method comprising using albumin and Japanese Patent Application Kokai (Laid-Open) No. 62-106,028 proposes a method comprising using a buffer composition as a stabilizer, respectively.

However, these methods are still unsatisfactory and, up to the present, an aqueous liquid preparation of thrombin has not yet been put to practical use.

The object of this invention is to provide a stable aqueous thrombin solution.

The present inventors have made extensive studies to achieve the above-mentioned object and resultantly found that thrombin can be kept stable even in its aqueous solution by using a sugar and an amino acid in combination as a stabilizer. After further studies based on the above finding, the present invention has been accomplished.

Thus, according to this invention, there is provided a stable aqueous thrombin solution containing thrombin and a sugar and an amino acid as a stabilizer.

The thrombin to be used in this invention is not particularly limited so long as it has a biological activity or a physiological activity. Examples of suitable thrombin include those obtained by fractionation of plasma protein. Thus, there may be used thrombin prepared by making thromboplastin, snake venom etc. act in the presence of Ca^{2+} on prothrombin purified from human or bovine plasma. There may also be used a commercially available pharmacopeial thrombin.

Thrombin to be used in this invention preferably has a specific activity in the neighborhood of 100 to 1,000 units/mg protein.

The concentration of thrombin in the thrombin- containing aqueous solution is preferably 50 to 5,000 units/ml, particularly preferably 100 to 3,000 units/ml.

The pH of the thrombin-containing aqueous solution is preferably 5 to 8, more preferably 6 to 7. To maintain such a pH, the use of buffer solutions is preferable. Examples of suitable buffer solutions include phosphate buffer solution and citrate buffer solution.

In this invention, a sugar and an amino acid are used in combination to heighten the stability of thrombin.

There is no particular restriction as to the sugar used as a stabilizer in this invention. Examples of suitable sugar are monosaccharides such as glucose and mannose; disaccharides such as maltose, sucrose and lactose; sugar alcohols such as sorbitol, mannitol and xylitol, which sugars may be used alone or in combination.

The quantity of sugar to be added is, for example, 1 to 20% (w/v), preferably 2 to 10% (w/v), for a solution containing 50 to 5,000 units of thrombin per milliliter.

The symbol "%(w/v)" means herein a percentage of a solute by weight per volume of solution.

The amino acid to be used in this invention is not particularly limited. Preferred examples thereof include neutral amino acids such as glycine, serine and threonine; acidic amino acids such as aspartic acid and glutamic acid; and basic amino acids such as arginine and lysine, which amino acids may be used alone or in combination.

The quantity of the amino acid to be added is, for example, 1 to 10% (w/v), preferably 1 to 5% (w/v), for a solution containing 50 to 5,000 units of thrombin per milliliter.

The stable aqueous thrombin solution of this invention may also be incorporated with other conventional additives.

The stabilizer comprising a sugar in combination with an amino acid used in this invention significantly stabilizes thrombin.

In this invention, an aqueous solution containing thrombin and the stabilizers may be prepared so as to meet the requirements described above by using known techniques and then subjected to dialysis, sterile filtration, dispensation into vials, etc. according to conventional techniques of preparation. Thus, a stabilized aqueous solution (medical preparation) of thrombin is obtained.

According to this invention, there is provided a stable aqueous thrombin solution (medical preparation) which can keep its appearance and properties satisfactorily even after a long-term storage.

This invention will be further described in more detail below with reference to the following Examples and Experimental Example. However, this invention is in no way limited by these Examples.

Example 1

Prothrombin was purified from normal human plasma by means of barium chloride adsorption and DEAE-Sephadex column chromatography [Bajaj, S.P. et al., J. Biol. Chem., 248, 7729(1973)]. The prothrombin obtained above was mixed with thromboplastin prepared from human placenta, human plasma and calcium chloride solution and subjected to thrombin conversion to give crude thrombin (thrombin activity per 1 mg protein: 10 units). The crude thrombin was purified by SP-Sephadex column chromatography [Lundblad, R. L., Biochemistry, 10, 2501 (1971)]. The purified thrombin was concentrated and then dialyzed against a 100 mM citrate buffer solution (pH 7.0) containing 7.5% of D-mannitol by ultrafiltration system (PELLICON®) to obtain a thrombin solution (3,500 units/ml, thrombin activity per 1 mg protein; 500 units).

To 45 ml of the thrombin solution obtained above were added 65 g of sucrose and 27 g of arginine [final concentration: thrombin 1,500 units/ml, sucrose 65% (w/v), arginine 27% (w/v)] and the resulting solution was adjusted to pH 5.9 with sodium hydroxide. The solution was heat-treated at 60°C for 20 hours and then dialyzed against 0.1M citrate buffer solution (pH 6.7) by ultrafiltration system (PELICON®). The dialyzed solution was concentrated and filtered to obtain 105 ml of an aqueous thrombin solution [containing 1,100 units/ml of thrombin, 7% (w/v) of sucrose and 4.7% (w/v) of arginine; pH 6.7].

The aqueous thrombin solution was stored at 5°C for one month. Then the appearance, thrombin activity, behavior in cellulose acetate membrane electrophoresis and gel filtration were examined. No marked change was observed in these test items, revealing that thrombin was stable in the test.

Example 2

Five thousand units of thrombin of official grade (Pharmacopeia of Japan) was dissolved in 10 ml of a phosphate buffer solution (pH 7). Then, sorbitol and glycine were added as a stabilizer to the resulting solution so that they have a concentration of 5% (w/v) and 1% (w/v), respectively, to obtain an aqueous thrombin solution.

This aqueous thrombin solution was also excellent in storage stability compared to that of Example 1.

Experimental Example 1

The aqueous thrombin solution obtained in Example 1 was used in order to examine the stability in a long-term storage. The aqueous thromtin solution was stored at 5°C for one month. Comparisons were made under 4 different stabilizing conditions, namely, no addition, sugar alone, amino acid alone,and both in combination. Table 1 shows the results of the examination.

Table 1

	Stabilizer		Percentage residual thrombin (%)
	Sucrose	Arginine	
After storage	-	-	5
	7% (w/v)	-	58
	-	4.6% (w/v)	46
	7% (w/v)	4.6% (w/v)	95
Before storage			100

Claims

1. A stable aqueous thrombin solution containing thrombin and a sugar and an amino acid as a stabilizer.

2. A stable aqueous thrombin solution according to Claim 1 which has a pH of 5 to 8.

3. A stable aqueous thrombin solution according to Claim 1 or 2, wherein the quantity of sugar is 1 to 20% (w/v) for a solution containing 50 to 5,000 units of thrombin per milliliter.

4. A stable aqueous thrombin solution according to Claim 1, 2 or 3, wherein the quantity of amino acid is 1 to 10% (w/v) for a solution containing 50 to 5,000 units of thrombin per milliliter.

5. A stable aqueous thrombin solution according to any preceding claim, wherein the sugar is at least one member selected from monosaccharides, disaccharides and sugar alcohols.

6. A stable aqueous thrombin solution according to any preceding claim, wherein the amino acid is at least one member selected from neutral amino acids, acidic amino acids and basic amino acids.

Claims for the following Contracting State : ES

1. A method for preparing a stable aqueous thrombin solution, characterized by adding a sugar and an amino acid as a stabilizer to an aqueous solution of thrombin.

2. A method for preparing a stable aqueous thrombin solution according to Claim 1, wherein the thrombin solution has a pH of 5 to 8.

3. A method for preparing a stable aqueous thrombin solution according to Claim 1 or 2, wherein the quantity of sugar is 1 to 20% (w/v) for a solution containing 50 to 5,000 units of thrombin per milliliter.

4. A method for preparing a stable aqueous thrombin solution according to Claim 1, 2 or 3, wherein the quantity of amino acid is 1 to 10% (w/v) for a solution containing 50 to 5,000 units of thrombin per milliliter.

5. A method for preparing a stable aqueous thrombin solution according to any preceding claim, wherein the sugar is at least one member selected from monosaccharides, disaccharides and sugar alcohols.

6. A method for preparing a stable aqueous thrombin solution according to any preceding claim, wherein the amino acid is at least one member selected from neutral amino acids, acidic amino acids and basic amino acids.